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Glutathione *S*-Transferase π in Colorectal Tumors Is Predictive for Overall Survival[1]

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ABSTRACT

Glutathione *S*-transferases (GSTs) are enzymes involved in the detoxification of xenobiotics and are divided into four subclasses, α, μ, π, and θ, with different although overlapping substrate specificities. Most human gastrointestinal tumors contain increased amounts of GST-π and GST enzyme activity. The relationship between GST parameters and tumor and patient characteristics, including overall survival, were studied retrospectively in 100 primary colorectal adenocarcinomas. Levels of GST-α, GST-μ, GST-π, and GST enzyme activity were not related to the Dukes stage, differentiation grade, localization, histological type and diameter of the tumor, or gender and age of the patient. Fifty-seven patients died (median survival, 21 months; range, 1–65 months) during follow-up, and 43 patients were still alive at the closing date of the study (median follow-up, 68 months; range, 60–87 months). Optimal dichotomization and uni- and multivariate analyses were done with the Cox proportional hazard model. Multivariate analysis with all clinicopathological parameters revealed higher Dukes stage (hazard ratio, 2.7; $P < 0.001$) and older age (hazard ratio, 2.8; $P = 0.001$) to be the only independent prognostic variables for overall survival. In contrast to GST-α and GST-μ, high levels of GST-π (hazard ratio, 3.1; $P = 0.002$) and GST enzyme activity (hazard ratio, 2.0; $P = 0.020$) in the tumors were found to have a significant prognostic value independent from the clinicopathological parameters when added separately to this Cox model. Thus, this study indicates that GST subclass levels in colorectal adenocarcinomas are not related to clinicopathological parameters and that the GST-π level and GST enzyme activity have a prognostic value for the overall survival of the patients.

INTRODUCTION

GSTs[3] are enzymes that catalyze the nucleophilic addition of glutathione to electrophilic centers of a wide variety of compounds. This reaction is the first step in the formation of mercapturic acids, a pathway resulting mostly in the elimination of potentially toxic compounds (1, 2). GSTs are also involved in the metabolism of several types of anticancer drugs (3) and are overexpressed in many human refractory tumors (4). On the basis of structural, physicochemical, enzymatic, and immunological properties, cytosolic GSTs are divided into three classes: α, μ, and π (2). Recently, a fourth class of GST (θ) has been described (5).

GST-π is the predominant subclass detected in colonic tissue in both normal mucosa and adenocarcinomas, whereas GST-μ and GST-α are present at much lower concentrations (6). Primary colorectal tumors contain higher amounts of GST-π and GST enzyme activity compared to normal mucosa, whereas the minor amounts of GST-α and GST-μ appear to be down-regulated within the tumor (6–9). Not only colorectal carcinomas but also tumors from stomach, urinary bladder, uterine cervix, and lung contain increased amounts of GST-π when compared to the adjacent normal tissue (4), suggesting that high levels of this GST subclass may offer some advantage to cancer cells. In a recent study, Gilbert *et al.* (10) described that high levels of GST-π in the tumor may be an important predictor of early recurrence and death in node-negative breast cancer patients. In the present study we examined the relationship of GST enzyme activity and the levels of GST classes α, μ, and π in primary colorectal adenocarcinomas with tumor and patient characteristics, including overall survival.

PATIENTS AND METHODS

Characteristics of Patients and Tumors. Specimens of histologically confirmed colorectal adenocarcinomas were obtained from 100 patients (58 men; median age, 68 years; range, 46–90 years; and 42 women, median age, 67 years; range, 44–89 years) who were operated on at the University Hospital Leiden during the period December 1983 through February 1988. A representative part of the tumor was selected by the pathologist and stored at −70°C. Tumors were classified according to Dukes (11) as modified by Astler and Coller (12) and Beart *et al.* (13) as follows: Dukes stage A, $n = 4$; B, $n = 50$; C, $n = 34$; or D, $n = 12$. Thirty-five carcinomas were located in the right side (from caecum to splenic flexure) and 65 were located in the left side (from splenic flexure to end of rectum) of the colorectum. Diameter, differentiation grade (54 poorly and 46 moderately/well differentiated), and histological type of the tumor (25 mucin producing and 75 non-mucin producing) were registered.

All patients received primary surgical therapy. They entered the study at the operation date and had a clinical follow-up for local recurrence and/or metastasis and survival for at least 5 years. Patient time experience ended in the event of death or at the closing date of the study, May 1993. Of the 100 patients included in the study, 57 died during the follow-up (median survival, 21 months; range, 1–65 months), and 31 patients (54%) had evidence of recurrence during that period. Forty-three patients were alive at the closing date of the study (median follow-up, 68 months; range, 60–87 months), and four (9%) of these had evidence of recurrent disease. Of the 35 patients with recurrent disease, 6 were operated on, another 6 received radiotherapy, and 4 were treated with anticancer drugs, whereas 19 patients received no additional anticancer treatment.

The study was approved by the local medical ethical review committee.

Quantification of GST Enzyme Activity and GST Subclasses. Cytosolic fractions were prepared as described before (6). Protein concentrations were determined according to Lowry *et al.* (14). GST enzyme activity was assayed by the method of Habig *et al.* (15) with the use of 1-chloro-2,4-dinitrobenzene as a substrate. Tumor cytosols were subjected to SDS-PAGE and subsequent Western blotting (6). Western blots were incubated with mAbs against GST-α, GST-μ, and GST-π, and the specific binding of the mAbs to their antigens was detected as described previously (6). Staining intensity was quantified by laser densitometry (Ultroscan XL, LKB, Bromma, Sweden) with the use of purified GSTs as marker proteins. The detection limit of this method is approximately 40 ng/mg protein, and within- and between-assay variation is 10–15%.

Statistical Analyses. Univariate and multivariate survival analyses were performed with the Cox's proportional hazard model (16) with the use of the EGRET statistical package (SERC Corp., Seattle, WA). All clinicopathological parameters studied were dichotomized. The cutoff points of age, diameter, and GST parameters were determined by increasing the value until the level of best discrimination was found with the use of Cox's univariate survival analysis, *i.e.*, optimal dichotomization. Multivariate survival analyses were performed by separately adding the GST variables to a model containing all clinicopathological parameters (localization, diameter, differentiation grade, histological type, and Dukes stage of the tumor, together with gender and age

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[3] The abbreviations used are: GST, glutathione *S*-transferase; HR, hazard ratio.

of the patient) in order to estimate their independent prognostic value in the overall survival. Overall survival curves were constructed by the method of Kaplan and Meier (17). Two by two tables obtained after dichotomization of both clinicopathological and GST parameters were tested with the use of the χ^2 test. Significance of the differences in GST activity and GST subclass levels between tumors, dichotomized according to clinicopathological parameters, were determined with the use of the Mann-Whitney U test. Besides dichotomization, parameters were also categorized into three or four subgroups, and significance of differences was evaluated with the use of the Kruskall-Wallis analysis. Associations between GST parameters were studied with the Spearman rank correlation procedure. All data are given as mean ± SEM. Statistical values of $P < 0.05$ were considered significant.

RESULTS

GST-α was detected in 22 of the 100 tumors examined, and 40 tumors were positive for GST-μ. GST-π was detected in all specimens investigated and was the major GST subclass in 97 tumor samples; only 3 tumors contained slightly more GST-μ. The distribution of the concentrations of GST subclasses and GST enzyme activity is shown in Fig. 1. Mean levels of GST-α, GST-μ, and GST-π were 0.09 ± 0.03, 0.25 ± 0.05, and 3.57 ± 0.20 μg/mg protein, respectively. Mean GST enzyme activity was 261 ± 13 nmol/min/mg protein.

When tumors were divided into two or more subgroups according to the clinicopathological parameters, no significant differences in GST subclass levels or GST enzyme activity were noticed (Table 1). There was no association between GST-μ and GST-α, and these two GST subclasses were not correlated with GST-π or GST enzyme activity. However, GST enzyme activity was correlated significantly ($r = 0.54$; $P < 0.001$) with GST-π level in the tumors.

Tumors from patients who survived had higher GST-μ and lower GST-α and GST-π levels, as well as lower GST enzyme activities, than tumors from patients who had died, but differences did not reach significance (Table 2). Univariate analysis of dichotomized patient and tumor characteristics showed that only the age of the patient (< *versus* >66.1 years; HR, 3.14; $P < 0.001$) and Dukes stage of the tumor (A + B *versus* C + D; HR, 2.71; $P < 0.001$) were associated significantly with overall survival (see Fig. 2, *A* and *B*, and Table 3). After determining the best discrimination point as cutoff level between survivors and nonsurvivors, a high GST-α (>0.14 μg/mg protein; HR, 2.40; $P = 0.008$; Fig. 2*C*) or a low GST-μ (<0.18 μg/mg protein; HR, 1.95; $P = 0.040$; Fig. 2*D*) level in the carcinoma appeared to be associated with significantly shorter overall survival. A high GST-π value (>5.30 μg/mg protein; HR, 1.92; $P = 0.054$) and a high GST enzyme activity (>243 nmol/min/mg protein; HR, 1.66; $P = 0.058$) also tended to be associated with poor prognosis, but only borderline significances were obtained in the univariate analyses (Table 4). The relationship between dichotomized clinicopathological

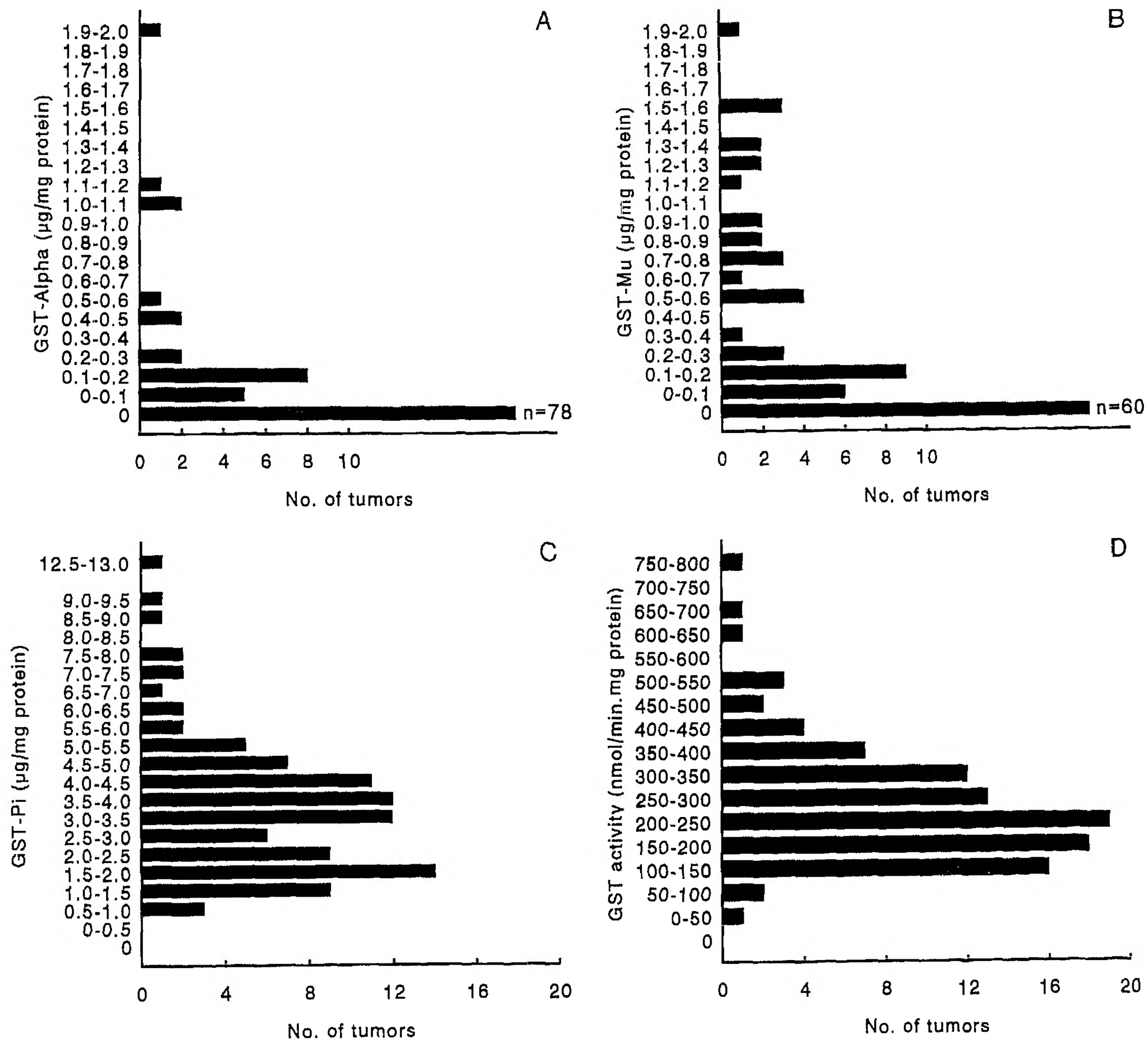

Fig. 1. Distribution of glutathione *S*-transferase class α (*A*), μ (*B*), and π (*C*) and glutathione *S*-transferase enzyme activity (*D*) in human primary colorectal carcinomas.

Table 1 *Glutathione S-transferase subclass composition and glutathione S-transferase enzyme activity in colorectal tumors dichotomized according to various clinicopathological parameters*[a]

Parameter dichotomized (*n*)	GST-α (μg/mg protein)	GST-μ (μg/mg protein)	GST-π (μg/mg protein)	GST enzyme activity (nmol/min/mg protein)
Gender				
Male (58)	0.11 ± 0.04[b]	0.18 ± 0.06	3.48 ± 0.26	256 ± 16
Female (42)	0.05 ± 0.03	0.33 ± 0.07	3.69 ± 0.33	268 ± 23
Age				
<66.1 yrs (43)	0.06 ± 0.04	0.29 ± 0.07	3.38 ± 0.28	266 ± 21
>66.1 yrs (57)	0.10 ± 0.04	0.21 ± 0.06	3.71 ± 0.29	256 ± 17
Localization				
Left (65)	0.08 ± 0.04	0.22 ± 0.06	3.53 ± 0.24	258 ± 16
Right (35)	0.10 ± 0.04	0.29 ± 0.08	3.65 ± 0.38	266 ± 23
Differentiation grade				
Poor (54)	0.05 ± 0.02	0.25 ± 0.06	3.80 ± 0.30	250 ± 18
Moderate/well (46)	0.13 ± 0.05	0.25 ± 0.07	3.30 ± 0.27	274 ± 20
Histological type				
Nonmucinous (75)	0.06 ± 0.02	0.25 ± 0.05	3.51 ± 0.23	271 ± 16
Mucinous (25)	0.16 ± 0.09	0.24 ± 0.09	3.77 ± 0.43	231 ± 24
Dukes stage				
A + B (54)	0.08 ± 0.04	0.25 ± 0.06	3.92 ± 0.31	278 ± 19
C + D (46)	0.09 ± 0.04	0.24 ± 0.06	3.17 ± 0.25	241 ± 17
Diameter				
≤4 cm (44)	0.10 ± 0.05	0.27 ± 0.08	3.47 ± 0.27	248 ± 18
>4 cm (56)	0.08 ± 0.03	0.23 ± 0.06	3.65 ± 0.30	271 ± 19

[a] No significant differences were noted.
[b] Mean ± SE.

and dichotomized GST parameters is depicted in Table 5. Except for a significantly higher percentage of females with tumors containing high levels of GST-μ (>0.18 μg/mg protein), no significant differences were noted. However, a relatively high percentage of patients younger than 66.1 years had tumors with high GST-α (>0.14 μg/mg protein) or low GST-μ (<0.18 μg/mg protein) concentrations, but differences were not significant.

In the multivariate Cox model, containing all clinicopathological parameters, Dukes stage (HR, 2.70; $P < 0.001$) and age (HR, 2.82; $P = 0.001$) were the only significant prognostic parameters (Table 3). When the GST parameters were added separately to the multivariate Cox analysis model containing all clinicopathological parameters, GST-π level (HR, 3.12; $P = 0.002$) and GST enzyme activity (HR, 1.97; $P = 0.020$) were significant prognostically, whereas GST-α (HR, 1.59; $P = 0.226$) and GST-μ (HR, 1.75; $P = 0.093$) lost their prognostic significance from the univariate analyses (see Fig. 2, *E* and *F*, and Table 4).

DISCUSSION

GST activity in normal colonic mucosa is mediated largely by GST-π; only minor levels of GST-α and GST-μ are found. When compared to the adjacent normal mucosa, GST-α and GST-μ are usually lower, whereas the levels of GST-π and GST enzyme activities are higher in colorectal tumors (6–9). In the current analysis, GST-α and GST-μ were detected in only 22 and 40% of the tumors, respectively, and concentrations were generally low. GST-π was the major GST subclass detected in almost all samples, and as a result, there was a significant linear correlation between GST-π level and GST enzyme activity in the tumors. The level of GST-μ and GST-α was not correlated significantly with the GST-π level, GST enzyme activity, or with each other.

Within the GST-μ family, one member, designated GSTM1–1, exhibits a genetic polymorphism. Due to this polymorphism, 40–50% of the western population has no functional *GSTM1* allele (18–21). In smokers, absence of GSTM1–1 has been associated with an increased risk of developing squamous cell carcinoma of the lung (22, 23), as well as with the development of urinary bladder (19, 24) or larynx cancer (24). Strange *et al.* (18) reported an increased risk of developing gastric or colorectal cancer in GSTM1–1-deficient individuals, illustrated by a group of 26 colorectal cancer patients, of whom 62% was GSTM1–1 deficient. Zhong *et al.* (21) reported that 56% of their 196 colorectal cancer patients was GSTM1–1 deficient, which was significantly more than the 42% detected in 225 controls. They also reported that the difference was much more pronounced in patients with tumors in the proximal colon (71% GSTM1–1 negative) than in the sigmoid colon or rectum (54% GSTM1–1 negative). In contrast, in the present study, 19 of the 35 carcinomas (54%) located in the right side of the colorectum, and 41 of the 65 carcinomas (63%) located in the left side of the colorectum were GSTM1–1 negative. Overall, 60 of the 100 patients with colorectal cancer were GSTM1–1 negative. In earlier studies we found that in different groups of colorectal cancer patients, 17 of the 50 (25) and 12 of the 24 (6) individuals lacked the GSTM1–1 isotype. From all 174 colorectal cancer patients analyzed by our group thus far, 89 (51%) were found to be GSTM1–1 negative. This figure is not different from the 40–50% reported for the general population (18–21). Thus, our data do not support the hypothesis that individuals with a GSTM1–1 deficiency have an increased risk of developing colorectal cancer.

Many studies have been performed to investigate clinical, pathological, genetic, and biochemical variables of colorectal adenocarcinomas with

Table 2 *Glutathione S-transferase subclass composition and GST enzyme activity of colorectal adenocarcinomas in relation to overall survival*[a]

	Alive (n = 43)	Dead (n = 57)
GST-α (μg/mg protein)	0.04 ± 0.03[b]	0.12 ± 0.04
GST-μ (μg/mg protein)	0.33 ± 0.08	0.18 ± 0.05
GST-π (μg/mg protein)	3.22 ± 0.26	3.84 ± 0.30
GST activity (nmol/min/mg protein)	252 ± 21	267 ± 17

[a] No significant differences were noted.
[b] Mean ± SE.

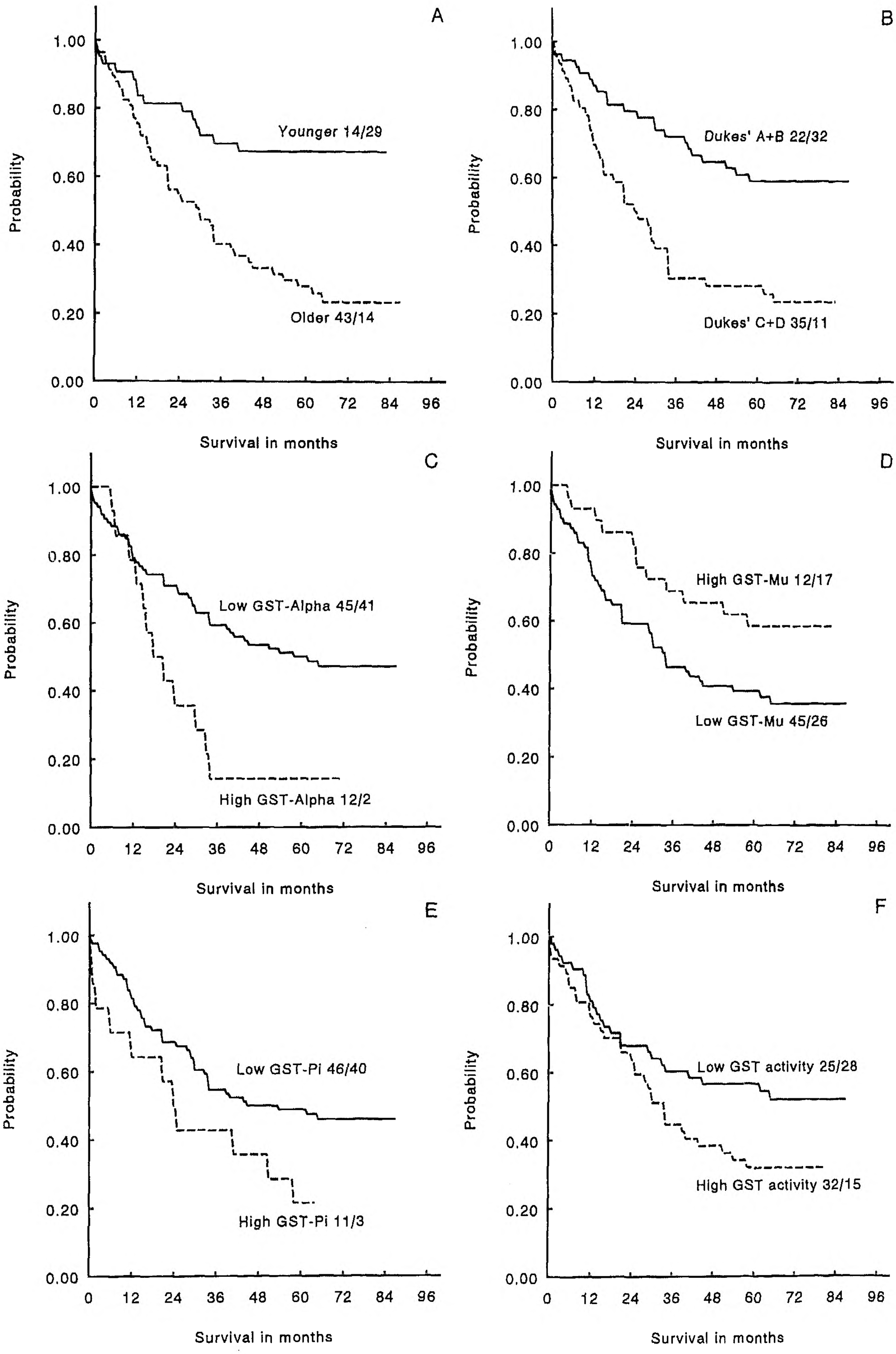

Fig. 2. Overall survival curves with patients dichotomized according to age (< *versus* >66.1 year; *A*) of the patients and Dukes stage (A + B *versus* C + D; *B*), GST-α level (< *versus* >0.14 μg/mg protein; *C*), GST-μ level (> *versus* <0.18 μg/mg protein; *D*), GST-π level ($\leq$ *versus* >5.30 μg/mg protein; *E*), and GST enzyme activity (< *versus* >243 nmol/min/mg protein; *F*) of the tumors. Values are the number of patients dead/alive at the end of the follow-up. For *P* values and hazard ratios see Tables 3 and 4.

Table 3 *Univariate and multivariate analyses of dichotomized clinicopathological parameters in relation to overall survival*

Parameter dichotomized (n)	No. of survivors (%)	Cox proportional hazard analysis (95% CI[a]; P) Univariate hazard ratio	Multivariate hazard ratio
Gender			
Male (58)	25 (43)		
Female (42)	18 (43)	1.07 (0.63–1.80; NS[b])	1.27 (0.73–2.23; NS)
Age			
<66.1 years (43)	29 (67)		
>66.1 years (57)	14 (25)	3.14 (1.71–5.76;<0.001)	2.82 (1.52–5.25;0.001)
Localization			
Left (65)	30 (46)		
Right (35)	13 (37)	1.22 (0.72–2.08; NS)	1.29 (0.72–2.30; NS)
Differentiation grade			
Poor (54)	24 (44)		
Moderate/well (46)	19 (41)	1.18 (0.70–1.99; NS)	1.36 (0.80–2.32; NS)
Histological type			
Nonmucinous (75)	34 (45)		
Mucinous (25)	9 (36)	1.50 (0.84–2.67; NS)	1.27 (0.69–2.31; NS)
Dukes stage			
A + B (54)	32 (59)		
C + D (46)	11 (24)	2.71 (1.58–4.65;<0.001)	2.70 (1.53–4.75;<0.001)
Diameter			
≤4 cm (44)	19 (43)		
>4 cm (56)	24 (43)	1.00 (0.59–1.69; NS)	1.13 (0.75–2.31; NS)

[a] CI, confidence interval.
[b] NS, not significant.

respect to their prognostic value in patients with this type of cancer (26–33). The most powerful clinicopathological prognostic factor in patients with colorectal carcinoma is still the extent of tumor spread (29, 34–37), often classified by the staging system proposed originally by Dukes (11–13). The association between survival and the Dukes stage of the tumor was confirmed in the present study; of all clinicopathological parameters incorporated in the overall survival model (patient gender and age, tumor stage, histological type, differentiation grade, localization, and diameter), only the Dukes stage of the tumor and the age of the patient were statistically significantly related to overall survival in both the univariate and the multivariate Cox proportional hazard analysis. We recently studied the prognostic relevance of plasminogen activators and their inhibitors in a similar, partially overlapping group of colorectal cancer patients (32). Results with respect to the prognostic value of clinicopathological parameters for overall survival were comparable to the findings of the present study; only Dukes stage and age were statistically significant. The finding that age was an important prognostic factor is in agreement several other studies (29, 34, 35), although the effect of age may have been augmented by the fact that we analyzed overall survival and not only cancer-related deaths.

Some studies have indicated that poor differentiation of colorectal carcinomas is associated with shorter survival (34, 36); however, this could not be confirmed in our relatively small group of patients. The histological type of the tumors also had no statistically significant prognostic value, but patients with a mucinous carcinoma seemed to have a slightly worse prognosis, which is in agreement with the results of Green *et al.* (38). With respect to the localization of the tumor, *i.e.*, left *versus* right sided, no differences in survival were noted, which is in agreement with results reported by others (36, 37).

No significant association of GST parameters with patient (age and gender) or tumor (stage, histological type, differentiation grade, localization, and diameter) characteristics were detected in the 100 tumors analyzed. Previous studies of breast tumors (10, 39), gastric carcinomas (40), and ovarian cancer (41) also failed to notice significant associations between clinicopathological factors and GST parameters.

Table 4 *Univariate and multivariate analyses of dichotomized glutathione S-transferase parameters in tumors in relation to overall survival*

Parameter dichotomized (*n*)	No. of survivors (%)	Cox proportional hazard analysis (95% CI[a]; P) Univariate hazard ratio	Adjusted hazard ratio[b]
GST-α (μg/mg protein)			
<0.14 (86)	41 (48)		
>0.14 (14)	2 (14)	2.40 (1.25–4.58;0.008)	1.59 (0.75–3.36; NS[c])
GST-μ (μg/mg protein)			
>0.18 (29)	17 (59)		
<0.18 (71)	26 (37)	1.95 (1.03–3.69;0.040)	1.75 (0.91–3.37; NS)
GST-π (μg/mg protein)			
≤5.30 (86)	40 (47)		
>5.30 (14)	3 (21)	1.92 (0.99–3.71;0.054)	3.12 (1.54–6.33;0.002)
GST activity (nmol/min/mg protein)			
<243 (53)	28 (53)		
>243 (47)	15 (32)	1.66 (0.98–2.81;0.058)	1.97 (1.11–3.51;0.020)

[a] CI, confidence interval.
[b] Adjustment was performed by adding the glutathione *S*-transferase parameters separately to a model containing all clinicopathological parameters (gender, age, localization, diameter, differentiation grade, histological type, and Dukes stage).
[c] NS, not significant.

Table 5 *Association between dichotomized glutathione S-transferase levels and dichotomized clinicopathological parameters*

Parameter dichotomized (*n*)	No. of tumors with GST-α <0.14 μg/mg protein (%)	No. of tumors with GST-μ >0.18 μg/mg protein (%)	No. of tumors with GST-π ≤5.30 μg/mg protein (%)	No. of tumors with GST activity <243 nmol/min/mg protein (%)
Gender				
Male (58)	49 (84)	12 (21)	51 (88)	33 (57)
Female (42)	37 (88)	17 (40)[a]	35 (83)	20 (48)
Age				
<66.1 years (43)	40 (93)	15 (35)	37 (86)	24 (56)
>66.1 years (57)	46 (81)	14 (25)	49 (86)	29 (51)
Localization				
Left (65)	57 (88)	17 (26)	55 (85)	37 (57)
Right (35)	29 (83)	12 (34)	31 (89)	16 (46)
Differentiation grade				
Poor (54)	49 (91)	15 (28)	45 (83)	33 (61)
Moderate/well (46)	37 (80)	14 (30)	41 (89)	20 (43)
Histological type				
Nonmucinous (75)	66 (88)	21 (28)	65 (87)	37 (49)
Mucinous (25)	20 (80)	8 (32)	21 (84)	16 (64)
Dukes stage				
A + B (54)	48 (89)	16 (30)	46 (85)	27 (50)
C + D (46)	38 (83)	13 (28)	40 (87)	26 (57)
Diameter				
≤4 cm (44)	38 (86)	13 (30)	38 (86)	24 (55)
>4 cm (56)	48 (86)	16 (29)	48 (86)	29 (52)

[a] Statistically significant *versus* male glutathione *S*-transferase μ ($P < 0.005$).

Previous studies have shown that most gastrointestinal tumors contain increased amounts of GST-π and GST enzyme activity when compared to adjacent normal mucosa (4, 6–9, 42, 43), suggesting that these elevated levels may offer a selective advantage to the tumor cells. In the present study we analyzed the prognostic relevance of the GST system to the overall survival of patients with colorectal adenocarcinomas. Comparing mean GST parameters of survivors with those of nonsurvivors, only minor differences were found. However, optimal dichotomization using the univariate Cox proportional hazard model revealed that patients with tumors containing a high level of GST-α or a low level of GST-μ had a significantly shorter survival. A high concentration of GST-π or a high GST enzyme activity also were associated with a shorter survival period, but the differences reached only borderline significance in the univariate analyses. In contrast, GST-π level and GST enzyme activity were significant prognostic parameters when added separately to a multivariate Cox model, which contained all clinicopathological parameters analyzed. Since GST-π was the major subclass detected in almost all samples analyzed, GST enzyme activity was correlated significantly with GST-π levels in the tumors. When GST-α and GST-μ levels were added to this multivariate Cox model, both had no significant independent prognostic value, probably because these two GST subclasses had a slight, nonsignificant association with age. These results indicate that in this group of patients, GST-π concentration and GST enzyme activity in the carcinoma are prognostic variables for the overall survival that are independent from the clinicopathological parameters, whereas GST-α and GST-μ are dependent prognostic variables.

GSTs are involved in the metabolism of several types of anticancer drugs (3). In addition, increased GST concentrations have been implicated as a resistance mechanism in cell lines selected for resistance toward various cytostatic drugs (4). Therefore, most studies concerning the possible prognostic value of tumoral GST levels on patient survival aimed at malignancies from patients who were treated subsequently with anticancer drugs. Studies by Wright *et al.* (44) and Peters *et al.* (39) in advanced breast cancer, Murphy *et al.* (45) and van der Zee *et al.* (46) in ovarium tumors, Joncourt *et al.* (47) in leukaemia, and Okuyama *et al.* (40) in gastric cancer did not show a significant correlation between pretreatment GST levels and response to chemotherapy or survival. In contrast, studies in ovarium tumors by Green *et al.* (48) and Hamada *et al.* (41) indicated that a low level of GST-π in the malignant tissue was prognostic for response to chemotherapy and prolonged survival, and Tidefeld *et al.* (49) reported a significant correlation between low GST-π expression at time of diagnosis and response to treatment in acute nonlymphoblastic leukaemia.

Moreover, Gilbert *et al.* (10) reported that a high GST-π level in the tumor was a significant predictor of early recurrence and death in node-negative breast cancer patients who received primary curative surgery without adjuvant chemotherapy. Recently, Grignon *et al.* (50) reported a possible prognostic value for GST-π levels in patients with renal cell carcinoma treated by radical nephrectomy only. Our study also indicates that GST-π level and GST enzyme activity in colorectal tumors may be of prognostic relevance for overall survival. These GST values may provide clinically useful prognostic parameters through which patients could be selected for adjuvant treatment.

It is not apparent from the present analysis why increased GST-π levels and GST enzyme activities in carcinomas are linked to a poorer prognosis in patients with colorectal cancer. The elevated levels of GST-π in most gastrointestinal carcinomas suggests that malignant cells are under a selective stress that favors cells with high GST-π content. However, it is also feasible that the GST isoenzymes themselves do not offer the selective advantage, but one or more of the GST genes are linked genetically or transcriptional to genes coding for other proteins that are involved in the clinical course of the disease, as suggested by Gilbert *et al.* (10). Further analysis of genes and proteins, the expression of which is linked to those of GSTs, may provide new ways to discriminate colorectal cancer patients with respect to their survival prognosis.

In conclusion, GST-π levels and GST enzyme activities in tumors of patients with colorectal adenocarcinoma are prognostic variables for overall survival independent from clinicopathological parameters and might form a basis for selective adjuvant therapy.

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